Cellular Senescence and the Senescence-Associated Secretory Phenotype in Human Synovium from Tissue Donors and Osteoarthritis Patients

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INTRODUCTION: Age remains an important risk factor for osteoarthritis (OA), but its role in promoting inflammatory processes relevant to OA remains unclear. Cellular senescence and the senescence-associated secretory phenotype (SASP), characterized by the production of proinflammatory cytokines and matrix degrading enzymes, are emerging as an important link between "inflammaging" and OA (1). Synovial inflammation contributes to OA pathogenesis and could promote cartilage degradation by the release of OA mediators including SASP factors. The purpose of this study was to investigate and compare cell senescence and SASP factor production using synovial tissue obtained from older adult tissue donors without a known history of OA and from patients undergoing knee replacement for advanced OA.

METHODS: Human knee synovial tissue was obtained from deceased adult tissue donors (n=9) without a known history of arthritis. The Collins grade of 0-4 was used to assess the donor knees for gross morphological changes of joint tissue degeneration. OA synovial tissue was obtained from patients undergoing total knee arthroplasty for advanced OA (n = 23). The use of human tissue was approved by institutional IRBs. A portion of tissue from each sample was enzymatically digested for isolation of synovial fibroblasts, and a second neighboring piece was processed for histology. Hematoxylin and eosin staining (H&E) was carried out to grade the microscopic structural changes in the synovium, including lining thickness, fibrosis, sub-synovial inflammatory cell infiltration, and angiogenesis (0-3 score for each feature) and were summed for a total score. Immunohistochemistry (IHC) and immunofluorescence (IF) techniques were employed to identify the presence of senescence markers (p16 and p21) and a SASP marker (IL-6) and were scored on a 0-3 scale. Grading was done blinded to other measures. Synovial fibroblasts isolated from the same synovial samples used for histology were treated with fibronectin fragments (FN-f), found in OA cartilage and synovial fluid (2), as a catabolic stimulus. Conditioned media was analyzed by ELISA for SASP factors IL-6, ENA-78 (CXCL5), and MMP-1. Results are shown as mean ± SD. Kolmogorv-Smirnov test was used to evaluate nonparametric data from two groups with unpaired donors. Spearman correlations were used to determine the relationships between different measured outcomes and synovial changes.

RESULTS: The tissue donors were slightly older (70.0 ± 5.7 yrs, range 60-78) than the OA patients (63.1 ± 8.9 yrs, range 42-79, p=0.02). The mean Collins grade for donor knees was 2.89 ± 0.8 (range 2-4), which indicates that on average the gross morphology was consistent with moderate "OA-like" changes. The summed synovial scores for tissue donors (3.25 ± 2.71) did not differ from OA samples (3.31 ± 1.46) nor did the sub-scores for individual features. Representative H&E and IHC images (IF not shown) are shown in Fig.1. There were no significant correlations in tissue donors between Collins grade and any of the measures; there were also no differences in levels of p16 or p21 between donor tissue and OA tissue. In donor tissues but not OA tissues, age was directly correlated to the IF score of senescence marker p16 (r = 0.98, p = 0.03, n = 5). Compared to tissue donors, synovial fibroblasts isolated from OA tissue produced significantly more basal MMP-1 (p=0.04) and more FN-f stimulated IL-6 (p=0.004) (Fig. 2). Interestingly, there was a strong negative correlation between the synovial fibrosis score and levels of IL-6 (r = -0.719, p= 0.035) and MMP-1 (r= -0.788, p = 0.015) produced by OA synovial fibroblasts isolated from the same tissue and stimulated with FN-f (Fig. 3).

DISCUSSION: Although the cadaveric donors did not have a known history of OA, the mean Collins grade of 2.89 on a scale of 0-4 and the lack of donors with a grade of 0 or 1 indicates the joints had moderate OA-like features, likely due to the advanced age of the donors. This, and the small sample size of tissue donors, limited the ability to find correlations between the Collins grade and the other measures. Increased levels with age of the senescence marker p16 in the donor tissues, and similar p16 and p21 levels in the donor and OA tissues, indicate synovial cell senescence was present in both. The biggest difference between the two tissue sources was the IL-6 response of isolated synovial fibroblasts to stimulation with the catabolic factor FN-f, which was significantly higher in the OA cells, as was basal production of MMP-1. The inverse relationship between the amount of fibrosis and levels of IL-6 and MMP-1 produced by FN-f stimulated OA synovial fibroblasts suggests that fibroblasts from more fibrotic tissue are less responsive to a catabolic stimulus, which could provide an unexpected protective benefit of fibrosis.

SIGNIFICANCE: Senescent cells in the knee synovium of older adults may precede the development of advanced symptomatic OA. The increased responsiveness of OA synovial fibroblasts to a catabolic stimulus found in OA cartilage and synovial fluid supports the paradigm of cross-talk between the cartilage and synovium in OA. How this may be modified by fibrosis in the synovium deserves further investigation.

REFERENCES: (1) Coryell et al, Nat Rev Rheumatol 2021 (2) Homandberg, Front Biosci 1999. **ACKNOWLEDGEMENTS**: Research was supported by NIA T35 AG038047, NIA RO1 AG044034, and the Rush Klaus Kuettner Chair for Osteoarthritis Research.

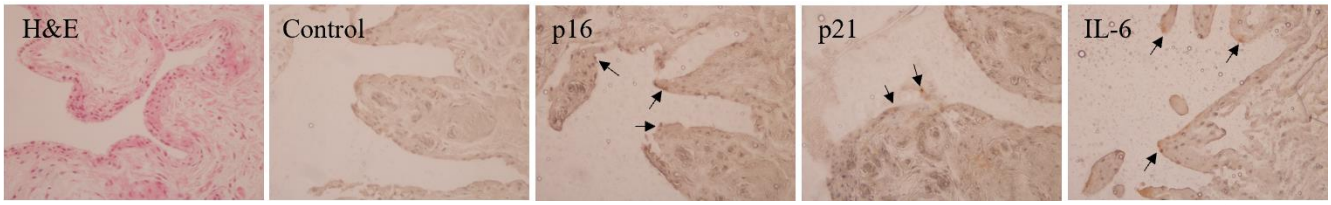

Fig. 1. Senescence and SASP markers in the synovium. Representative sections from OA patient (H&E) and tissue donor (IHC). Control=no 1^0 antibody.

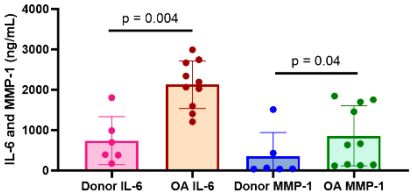

Fig. 2. IL-6 and MMP-1 levels produced by donor and OA synovial fibroblasts in response to FN-f stimulation.

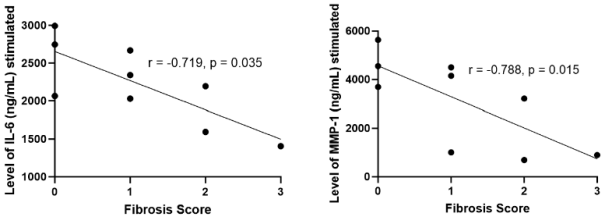

Fig. 3: Correlation of fibrosis score and SASP factors IL-6 and MMP-1.